

# Real-time PCR Detection of *E. coli* O157:H7 in Food Samples

## USER GUIDE

Automated DNA isolation using magnetic bead-based technology

for use with:

PrepSEQ™ Nucleic Acid Extraction Kit

KingFisher™ Flex Purification System with 96 Deep-Well Head

MagMAX™ Express-96 Deep Well Magnetic Particle Processor

MicroSEQ™ *E. coli* O157:H7 Detection Kit

Applied Biosystems™ 7500 Fast Real-Time PCR Instrument

RapidFinder™ Express Software v2.0 or later

**Catalog Numbers** 4427409, 4428176, 4445656, 4480466

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For testing of Food and Environmental samples only.

**ThermoFisher**  
S C I E N T I F I C

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Revision	Date	Description
B	14 August 2024	<ul style="list-style-type: none"><li>• Troubleshooting was added for possible instance of varying morphology of PCR pellets.</li><li>• The software version was updated for RapidFinder™ Express Software.</li><li>• Characteristics were added for the 7500 Fast Real-Time PCR Instrument.</li></ul>
A.0	12 November 2018	<p>New document for the <i>Real-time PCR Detection of E. coli O157:H7 in Food Samples User Guide (Automated DNA Isolation; AOAC)</i>.</p> <ul style="list-style-type: none"><li>• Includes the complete AOAC Research Institute <i>Performance Tested Methods</i>™ -certified workflow that covers enrichment, automated DNA isolation, and real-time PCR detection.</li><li>• Supersedes:<ul style="list-style-type: none"><li>– <i>PrepSEQ™ Nucleic Acid Extraction Kit User Guide</i> (Pub. No. 4426513)</li><li>– <i>MicroSEQ™ E. coli O157:H7 Detection Kit User Guide</i> (Pub. No. 4426511)</li></ul></li></ul>

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**IMPORTANT!** Before using these products, read and understand the information in the "Safety" appendix in this document.

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## General overview

This guide describes the following AOAC Research Institute *Performance Tested Methods*<sup>SM</sup>-certified workflow for detection of *E. coli* O157:H7 in raw beef and produce samples:

1. Enrichment of food samples by one of the following methods (see "Overview of the enrichment and DNA isolation options"):
  - 25 g or 25 mL samples in prewarmed Brain Heart Infusion (BHI) Broth (Workflow A)
  - 25 g or 25 mL samples in Buffered Peptone Water (BPW) (Workflow B)
  - 375 g samples in BPW (workflow C)
2. Automated preparation of PCR-ready DNA using the PrepSEQ™ Nucleic Acid Extraction Kit and one of the following magnetic particle processors, for high-throughput sample processing in a 96-well format with minimal handling.
  - MagMAX™ Express-96 Magnetic Particle Processor
  - KingFisher™ Flex Purification System with 96 Deep-Well Head
3. Real-time PCR detection of *E. coli* O157:H7 DNA using the MicroSEQ™ *E. coli* O157:H7 Detection Kit and RapidFinder™ Express Software v2.0 or later on the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument.
4. Confirmation testing of positive samples.  
See Chapter 6, "Recommended confirmation methods" for detailed information.

This workflow is intended for use by microbiological analysts who need to test for *E. coli* O157:H7 in food samples. These kits are for use in food testing only. Not for any animal or human therapeutic or diagnostic use.

Go to [thermofisher.com/foodsafety](https://www.thermofisher.com/foodsafety) for a list of workflows for detection of *E. coli* (Pub. No. MAN0009419).

## Overview of the enrichment and DNA isolation options

Select an enrichment and DNA isolation workflow based on the sample amount, your preferred media and enrichment time, and processing instrument. See “AOAC Performance Tested Methods<sup>SM</sup> Certification” on page 40 for validated sample matrices.

**Table 1 Workflow options for the KingFisher™ Flex-96 instrument**

Enrichment media	Enrichment time <sup>[1]</sup>	Volume <sup>[2]</sup>	Food type/PK requirement <sup>[3]</sup>	KingFisher™ Flex-96 script
<b>Workflow A, 25 g or 25 mL food samples</b>				
Prewarmed (42±1°C) Brain Heart Infusion (BHI) Broth	All foods except juices: 6–8 hr <sup>[4]</sup> Juices: 8–10 hr <sup>[4]</sup>	1 mL <sup>[5]</sup>	Animal products <sup>[6]</sup> : with PK	4445656PrepSEQ_APK
			Non-animal products: without PK	4445656PrepSEQ_A
<b>Workflow B, 25 g or 25 mL food samples</b>				
Buffered Peptone Water (BPW)	16–20 hr	200 µL <sup>[7]</sup>	Animal products <sup>[6]</sup> : with PK	4445656PrepSEQ_BPK
			Non-animal products: without PK	4445656PrepSEQ_B
<b>Workflow C, 375 g food samples</b>				
BPW	16–20 hr	1 mL <sup>[5]</sup>	All foods: with PK	4445656PrepSEQ_CPK

<sup>[1]</sup> All enrichments are incubated at 42±1°C.

<sup>[2]</sup> Volume of enriched sample for DNA isolation

<sup>[3]</sup> PK: Proteinase K

<sup>[4]</sup> For convenience, samples can be enriched in BHI for up to 16 hours.

<sup>[5]</sup> Pellet and resuspend in appropriate buffer.

<sup>[6]</sup> Animal products include ground beef and beef trim.

<sup>[7]</sup> Transfer directly to deep-well plate; no bacterial pellet required.

**Table 2 Workflow options for the MagMAX™ Express-96 instrument**

Enrichment media	Enrichment time <sup>[1]</sup>	Volume <sup>[2]</sup>	Food type/PK requirement <sup>[3]</sup>	MagMAX™ Express-96 script
<b>Workflow A, 25 g or 25 mL food samples</b>				
Prewarmed (42±1°C) Brain Heart Infusion (BHI) Broth	All foods except juices: 6–8 hr <sup>[4]</sup> Juices: 8–10 h <sup>[4]</sup> r	1 mL <sup>[5]</sup>	Animal products <sup>[6]</sup> : with PK	44000799DWPRepSEQGP
			Non-animal products: without PK	44000799DWPRepSEQGN

Enrichment media	Enrichment time <sup>[1]</sup>	Volume <sup>[2]</sup>	Food type/PK requirement <sup>[3]</sup>	MagMAX™ Express-96 script
<b>Workflow B, 25 g or 25 mL food samples</b>				
Buffered Peptone Water (BPW)	16–20 hr	200 µL <sup>[7]</sup>	Animal products <sup>[6]</sup> : with PK	44000799DWPRepSEQPK
			Non-animal products: without PK	44000799DWPRepSEQDL
<b>Workflow C, 375 g food samples</b>				
BPW	16–20 hr	1 mL <sup>[5]</sup>	All foods: with PK	44000799DWPRepSEQGP

<sup>[1]</sup> All enrichments are incubated at 42±1°C.

<sup>[2]</sup> Volume of enriched sample for DNA isolation

<sup>[3]</sup> PK: Proteinase K

<sup>[4]</sup> For convenience, samples can be enriched in BHI for up to 16 hours.

<sup>[5]</sup> Pellet and resuspend in appropriate buffer.

<sup>[6]</sup> Animal products include ground beef and beef trim.

<sup>[7]</sup> Transfer directly to deep-well plate; no bacterial pellet required.

## Required materials

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or [thermofisher.com](https://www.thermofisher.com). They may also be available through Fisher Scientific ([fisherscientific.com](https://www.fisherscientific.com)), MLS, or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

**Note:** Parts may ship separately depending on configuration and storage conditions.

## Materials for enrichment of food samples

**Table 3** Equipment, accessories, and consumables

Item	Source
Incubator, 42±1°C	<a href="https://www.thermofisher.com">thermofisher.com</a>
<b>Homogenizer laboratory blender or diluter, one of the following or equivalent:</b>	
<ul style="list-style-type: none"> <li>Homogenizer Laboratory Blender</li> <li>Diluflex™ Pro Automated Gravimetric Dilutor with simple (non-robotic) dispensing arm</li> <li>Diluflex™ Pro Automated Gravimetric Dilutor with robotic dispensing arm</li> </ul>	<a href="#">DB5000A</a> <a href="#">DB4100A</a> <a href="#">DB4150A</a>
<b>Homogenizer bag appropriate for the sample type</b>	
Homogenizer bag, with mesh, 6" × 9", 24 oz (Whirl-Pak™ Filter Bag for Homogenizer Blenders, or equivalent)	Nasco #B01348WA or equivalent



**Table 3** Equipment, accessories, and consumables (continued)

Item	Source
Homogenizer bag, 6" × 9", 24 oz (Whirl-Pak™ Sample Bag, or equivalent)	Nasco #B01297WA or equivalent
For 375 g samples: Homogenizer bag, with mesh, 10" × 15", 92 oz (Whirl-Pak™ Filter Bag for Homogenizer Blenders, or equivalent)	Nasco #B01488WA or equivalent
<b>Enrichment media appropriate for the sample and workflow chosen</b>	
Brain Heart Infusion (BHI) Broth (workflow A)	One of the following or equivalent: <ul style="list-style-type: none"> <li>Oxoid™ <a href="#">CM1135B</a></li> <li>Remel™ <a href="#">R060270</a></li> </ul>
Buffered Peptone Water (BPW) (workflows B and C)	Oxoid™ <a href="#">CM1211B</a> , <a href="#">CM1049B</a> or equivalent

## Materials for DNA isolation

**Table 4** PrepSEQ™ Nucleic Acid Extraction Kit

Contents	Cat. No. <a href="#">4480466</a> (100 reactions)	Cat. No. <a href="#">4428176</a> (300 reactions)	Storage <sup>[1]</sup>
Lysis Buffer	2 × 50 mL	6 × 50 mL	15°C to 30°C
Magnetic Particles	2 × 1.5 mL	6 × 1.5 mL	
Binding Solution (Isopropanol) <sup>[2]</sup>	1 empty bottle	3 empty bottles	
Wash Buffer Concentrate <sup>[3]</sup>	2 × 26 mL	6 × 26 mL	
Elution Buffer	25 mL	3 × 25 mL	
Proteinase K (PK) Buffer	50 mL	3 × 50 mL	-25°C to -15°C
Proteinase K, 20 mg/mL	1.25 mL	3 × 1.25 mL	

<sup>[1]</sup> See the expiration date on the box.

<sup>[2]</sup> Add ~35 mL of 100% isopropanol to the empty bottle before use.

<sup>[3]</sup> Add 74 mL of 95% ethanol before use.

**Table 5** Magnetic particle processor

Item	Source
<b>KingFisher™ Flex-96 instrument and accessories</b>	
KingFisher™ Flex Purification System with 96 Deep-Well Head	<a href="#">A32681</a> , 96 deep-well plate, or equivalent <sup>[1]</sup>
KingFisher™ 96 Deep-Well Plate, V-bottom	<a href="#">95040450</a>
KingFisher™ 96 KF microplates (200 µL)	<a href="#">97002540</a>

Item	Source
KingFisher™ Flex 96 heating block	<a href="#">24075420</a>
KingFisher™ 96 tip comb for deep-well magnets	<a href="#">97002534</a>
Finntip™ Filtered Pipette Tips	<a href="#">94052320</a> or equivalent
<b>MagMAX™ Express-96 instrument and accessories</b>	
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	Contact your local sales representative.
MagMAX™ Express-96 Deep Well Plates	<a href="#">4388476</a>
MagMAX™ Express-96 Standard Plates	<a href="#">4388475</a>
MagMAX™ Express-96 Deep Well Tip Combs	<a href="#">4388487</a>

[1] For the KingFisher™ Flex instrument, 96 plate with standard magnetic head (Cat. No. [5400620](#)), the 96 deep-well magnetic head is required (Cat. No. [24074430](#)).

**Table 6 Other materials not included in the PrepSEQ™ Nucleic Acid Extraction Kit**

Item	Source
<b>Equipment</b>	
96-Well Magnetic-Ring Stand	<a href="#">AM10050</a>
Block heater, 37°C	MLS
Laboratory mixer (vortex or equivalent)	MLS
Pipettors: <ul style="list-style-type: none"> <li>• Positive-displacement</li> <li>• Air-displacement</li> <li>• Multichannel</li> </ul>	MLS
<i>(Optional, but recommended)</i> Plate centrifuge	MLS
<b>Consumables</b>	
Disposable gloves	MLS
Micropipette tips, aerosol-resistant	MLS
<i>(Optional)</i> MicroAmp™ Clear Adhesive Film	<a href="#">4306311</a>
<b>Reagents</b>	
Ethanol, 95%	MLS
Isopropanol, 100%	MLS
Nuclease-Free Water (not DEPC-Treated)	<a href="#">AM9938</a>

## Materials for PCR detection

Table 7 MicroSEQ™ E. coli O157:H7 Detection Kit (96 reactions; Cat. No. [4427409](#), [4445656](#))

Contents [1]	Amount	Cap color	Storage
E. coli O157:H7 Assay Beads, 8-tube strips	12 strips (96 tubes) 1 rack	Orange (rack)	5±3°C Protect from light and moisture.[2]
MicroAmp™ Optical 8-Cap Strips	12 strips (96 caps)	N/A	
Pathogen Detection Negative Control[3]	1.5 mL	Red	5±3°C

[1] Cat. No. [4445656](#) includes the PrepSEQ™ Nucleic Acid Extraction Kit.

[2] Excessive exposure to light may affect the fluorescent probes. To protect the beads from moisture, do not remove the desiccant from the pouch, and seal the pouch tightly each time you remove assay bead strips.

[3] The Pathogen Detection Negative Control is included in a separate box and may be shipped separately.

Item	Source
<b>Instruments and equipment</b>	
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument	A30304 (desktop) A30299 (laptop) Contact your local microbiology sales representative.
RapidFinder™ Express Software v2.0 or later	Download the latest version at <a href="http://thermofisher.com/rapidfinder-express-software">thermofisher.com/rapidfinder-express-software</a>
7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips	<a href="#">A29252</a>
MicroAmp™ 96-Well Base	<a href="#">N8010531</a>
MicroAmp™ Cap Installing Tool	<a href="#">4330015</a>
MicroAmp™ Multi-removal Tool	<a href="#">4313950</a>
Benchtop microcentrifuge with 8-tube strip adapter <i>or</i> Plate centrifuge	MLS
Laboratory mixer (vortex mixer or equivalent)	MLS

Item	Source
Pipettors: <ul style="list-style-type: none"> <li>• Positive-displacement</li> <li>• Air-displacement</li> <li>• Multichannel</li> </ul>	MLS
<b>Consumables</b>	
Aerosol-resistant pipette tips	MLS
Disposable gloves	MLS
MicroAmp™ Fast 8-Tube Strip, 0.1-mL <sup>[1]</sup>	<a href="#">4358293</a>
MicroAmp™ Optical 8-Cap Strip, 300 strips <sup>[1]</sup>	<a href="#">4323032</a>
<b>Reagents</b>	
Nuclease-Free Water (not DEPC-Treated)	<a href="#">AM9938</a>

<sup>[1]</sup> Required to evenly distribute the clamping load applied to the tube strips during PCR processing. Do not use other tube strips, which could result in crushed tubes.

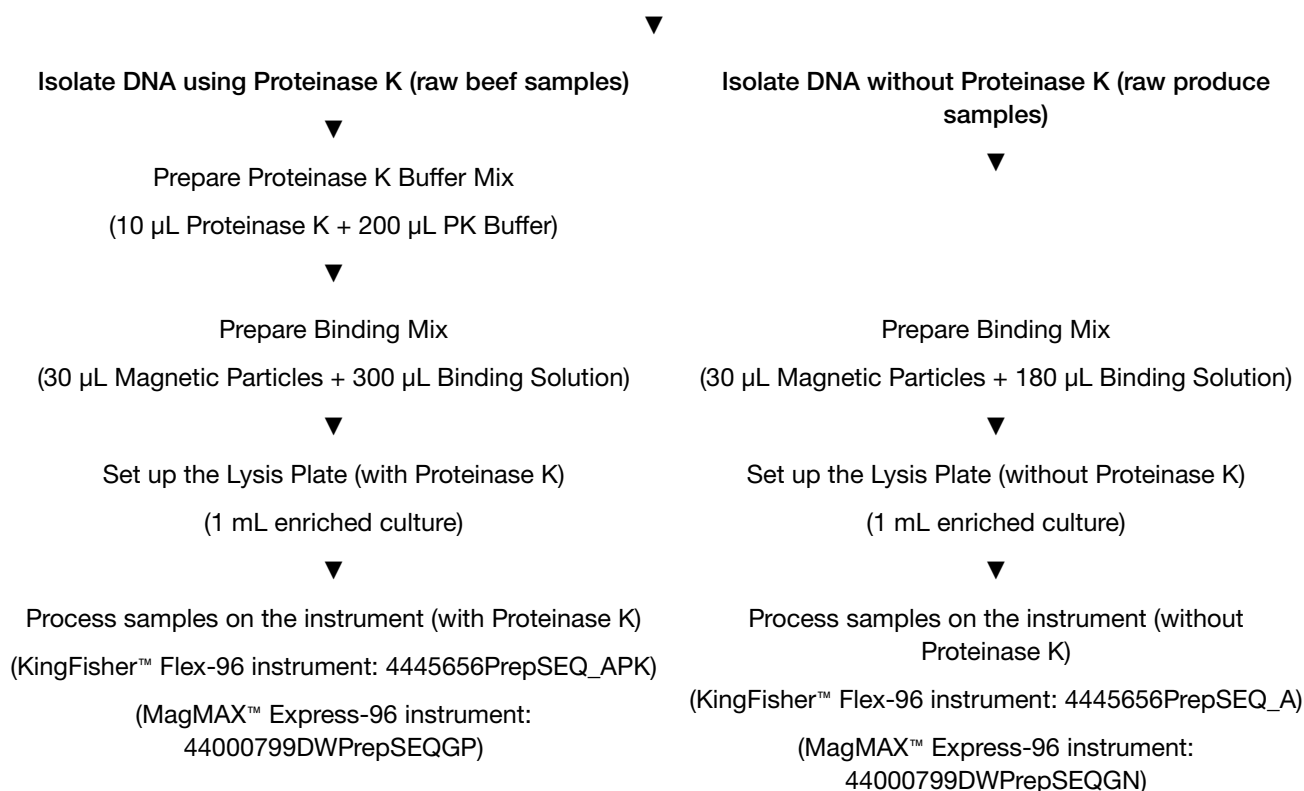
# 2

## Enrich 25 g or 25 mL of food sample in BHI and isolate DNA (workflow A)

### Workflow

#### Enrich food samples in BHI Broth

(25 g or 25 mL sample, 225 mL prewarmed BHI, 42±1°C, see “Overview of the enrichment and DNA isolation options” on page 7 for incubation times)



## Important procedural guidelines for enrichment and DNA isolation

### Guidelines for sample enrichment

- Use proper aseptic technique while handling samples to avoid cross-contamination.
- Use a forced air incubator and ensure sufficient space between enrichment bags to allow for air flow.

### For high-fat samples: remove fat layer before lysis

For samples that contain a distinct, top, fat layer following centrifugation, remove the fat layer and supernatant as follows:

Type of fat layer	Fat layer and supernatant removal
Liquid	<ol style="list-style-type: none"> <li>1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet.</li> <li>2. Continue to collect supernatant from the top surface until all the supernatant is removed.</li> <li>3. Discard the supernatant into a waste container.</li> </ol>
Solid	<ol style="list-style-type: none"> <li>1. Use a pipette tip to gently dislodge the fat layer without disturbing the pellet.</li> <li>2. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed.</li> <li>3. Discard the supernatant into a waste container.</li> </ol>

## Enrich food samples in BHI Broth

1. Prepare 225 mL of Brain Heart Infusion (BHI) Broth for each 25 g or 25 mL of food sample, according to the instructions of the manufacturer, and prewarm to  $42\pm 1^\circ\text{C}$ .
2. Combine the food sample with 225 mL of prewarmed BHI Broth in a homogenizer bag, and homogenize.

A filtered bag may be used for enrichment of samples with particulates.

Sample type	Method
Coarse food types	Homogenize the sample thoroughly using a laboratory blender. Hand massage foods that cannot be processed in a homogenizer: squeeze the bag 5–10 times.
Liquids	Mix by hand.

3. Incubate the sample at  $42\pm 1^\circ\text{C}$  under static conditions:

Sample type	Enrichment time
All foods except juices	6–8 hours
Juices	8–10 hours

For convenience, the sample may be enriched in BHI for up to 16 hours.

## Before first use of the kit

### Prepare Binding Solution and Wash Buffer

Before using a new PrepSEQ™ Nucleic Acid Extraction Kit, prepare the reagents:

- **Binding Solution**—Add approximately 35 mL of 100% isopropanol to an empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer**—Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, then mix well. Label the bottle to indicate that ethanol is added.

## Before each use of the kit

### Resuspend Magnetic Particles

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**IMPORTANT!** Mix the particles vigorously before each use to ensure that all salts are dissolved.

White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is fully dissolved prior to use.

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1. Incubate the tube of Magnetic Particles at  $37\pm 1^\circ\text{C}$  for approximately 10 minutes.
2. Vortex for approximately 10 seconds.

---

**Note:** If the white precipitate is not completely dissolved after 10 minutes at  $37^\circ\text{C}$ , apply longer incubation times and higher temperatures (up to  $50^\circ\text{C}$ ).

---

3. Keep at room temperature ( $23\pm 5^\circ\text{C}$ ) until ready for use.

## Set up the processing plates

Set up the processing plates as described in the following table.

Plate	Plate type	Action
Tip Comb	Standard	Place a 96-well deep-well tip comb in a standard plate.
Elution Plate	Standard	Add 140 $\mu\text{L}$ of Elution Buffer to each sample and control well.
Wash Plate 1	Deep-Well	Add 300 $\mu\text{L}$ of Wash Buffer to each sample and control well.
Wash Plate 2	Deep-Well	Add 300 $\mu\text{L}$ of Wash Buffer to each sample and control well.

## Isolate DNA using Proteinase K (raw beef samples)

### Prepare Proteinase K Buffer Mix

- Combine the following components for the number of samples required.

Component	Volume per sample	Volume for $n$ samples <sup>[1]</sup>
Proteinase K, 20 mg/mL	10 $\mu\text{L}$	11 $\mu\text{L} \times n$
Proteinase K (PK) Buffer	200 $\mu\text{L}$	220 $\mu\text{L} \times n$

<sup>[1]</sup> Includes 10% overage.

- Mix well, and use immediately or store on ice until ready to use.

### Prepare Binding Mix

Prepare Binding Mix just before use.

Combine the following components for the number of extractions required, and mix well by vortexing for approximately 10 seconds.

Component	Volume per extraction	Volume for $n$ samples <sup>[1]</sup>
Binding Solution (isopropanol)	300 $\mu\text{L}$	330 $\mu\text{L} \times n$
Magnetic Particles <sup>[2]</sup>	30 $\mu\text{L}$	33 $\mu\text{L} \times n$
<b>Total volume per extraction</b>	<b>330 <math>\mu\text{L}</math></b>	<b>363 <math>\mu\text{L} \times n</math></b>

<sup>[1]</sup> Includes 10% overage.

<sup>[2]</sup> Resuspended and thoroughly mixed.



## Set up the Lysis Plate (with Proteinase K)

1. Transfer 1 mL of enriched culture to a 1.5-mL microcentrifuge tube, then centrifuge the tube at 12,000–16,000 × *g* for about 3 minutes.
2. Remove and discard the supernatant as quickly as possible to prevent dissipation of the pellet.

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**Note:** If no pellet is visible after centrifugation (for example, as found in filtered juices), leave ~50 µL of supernatant in the tube to avoid aspiration the bacterial pellet.

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3. (Optional) If necessary, follow “For high-fat samples: remove fat layer before lysis” on page 14.
4. Add 210 µL of Proteinase K Buffer Mix to the pellet, and mix well to resuspend the pellet.
5. Transfer the sample to a deep well Lysis Plate.
6. (Optional but recommended) Set up a negative extraction control (NEC) well containing 210 µL of nuclease-free water.

## Process samples on the instrument (with Proteinase K)

1. Select the program on the instrument, and press **Start**.

Instrument	Program
KingFisher™ Flex-96	4445656PrepSEQ_APK
MagMAX™ Express-96	44000799DWPRepSEQGP

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the Tip Comb, then press <b>Start</b> .
Elution Plate	Load the Elution Plate, then press <b>Start</b> .
Wash Plate 2	Load Wash Plate 2, then press <b>Start</b> .
Wash Plate 1	Load Wash Plate 1, then press <b>Start</b> .
Lysis Plate	Load the Lysis Plate, then press <b>Start</b> .

3. Dispense 300 µL of Lysis Buffer when prompted by the instrument (after 20 minutes).
  - a. Remove the Lysis Plate and add 300 µL of Lysis Buffer to each sample and control well.
  - b. Load the Lysis Plate into the instrument, and press **Start**.
4. Dispense 330 µL of Binding Mix when prompted by the instrument (after 18 minutes).
  - a. Vortex the Binding Mix for 5–10 seconds to ensure uniform distribution of the Magnetic Particles.
  - b. Remove the Lysis Plate and add 330 µL of Binding Mix to each sample and control well.

- c. Load the Lysis Plate into the instrument and press **Start**.

The remainder of the procedure (~30 minutes) is automated and does not require further user interaction.

5. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.

Proceed directly to real-time PCR. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film and store the DNA in one of the following ways:

- At  $5\pm 3^{\circ}\text{C}$  for up to 24 hours.
- Below  $-18^{\circ}\text{C}$  for up to 1 year.

If required, validate storage of the DNA according to EN ISO 20837:2006.

## Isolate DNA without Proteinase K (raw produce samples)

### Prepare Binding Mix

Prepare Binding Mix just before use.

Combine the following components for the number of extractions required, and mix well by vortexing for approximately 10 seconds.

Component	Volume per extraction	Volume for $n$ samples <sup>[1]</sup>
Binding Solution (isopropanol)	180 $\mu\text{L}$	198 $\mu\text{L} \times n$
Magnetic Particles <sup>[2]</sup>	30 $\mu\text{L}$	33 $\mu\text{L} \times n$
<b>Total volume per extraction</b>	<b>210 <math>\mu\text{L}</math></b>	<b>231 <math>\mu\text{L} \times n</math></b>

<sup>[1]</sup> Includes 10% overage.

<sup>[2]</sup> Resuspended and thoroughly mixed.

### Set up the Lysis Plate (without Proteinase K)

1. Transfer 1 mL of enriched culture to a 1.5-mL microcentrifuge tube, then centrifuge the tube at 12,000–16,000  $\times g$  for about 3 minutes.
2. Remove and discard the supernatant as quickly as possible to prevent dissipation of the pellet.

---

**Note:** If no pellet is visible after centrifugation (for example, as found in filtered juices), leave ~50  $\mu\text{L}$  of supernatant in the tube to avoid aspiration the bacterial pellet.

---

3. (Optional) If necessary, follow “For high-fat samples: remove fat layer before lysis” on page 14.
4. Add 300  $\mu\text{L}$  of Lysis Buffer to the pellet, and mix well to resuspend the pellet.

5. Transfer the sample to a deep well Lysis Plate.
6. (Optional but recommended) Set up a negative extraction control (NEC) well containing 300 µL of Lysis Buffer.

## Process samples on the instrument (without Proteinase K)

1. Select the program on the instrument, and press **Start**.

Instrument	Program
KingFisher™ Flex-96	4445656PrepSEQ_A
MagMAX™ Express-96	44000799DWPRepSEQGN

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the Tip Comb, then press <b>Start</b> .
Elution Plate	Load the Elution Plate, then press <b>Start</b> .
Wash Plate 2	Load Wash Plate 2, then press <b>Start</b> .
Wash Plate 1	Load Wash Plate 1, then press <b>Start</b> .
Lysis Plate	Load the Lysis Plate, then press <b>Start</b> .

3. Dispense 210 µL of Binding Mix when prompted by the instrument (after 18 minutes).
  - a. Vortex the Binding Mix for 5–10 seconds to ensure uniform distribution of the Magnetic Particles.
  - b. Remove the Lysis Plate from the instrument, and add 210 µL of Binding Mix to each sample and control well.
  - c. Load the Lysis Plate into the instrument and press **Start**.

The remainder of the procedure (~30 minutes) is automated and does not require further user interaction.

4. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.

Proceed directly to real-time PCR. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film and store the DNA in one of the following ways:

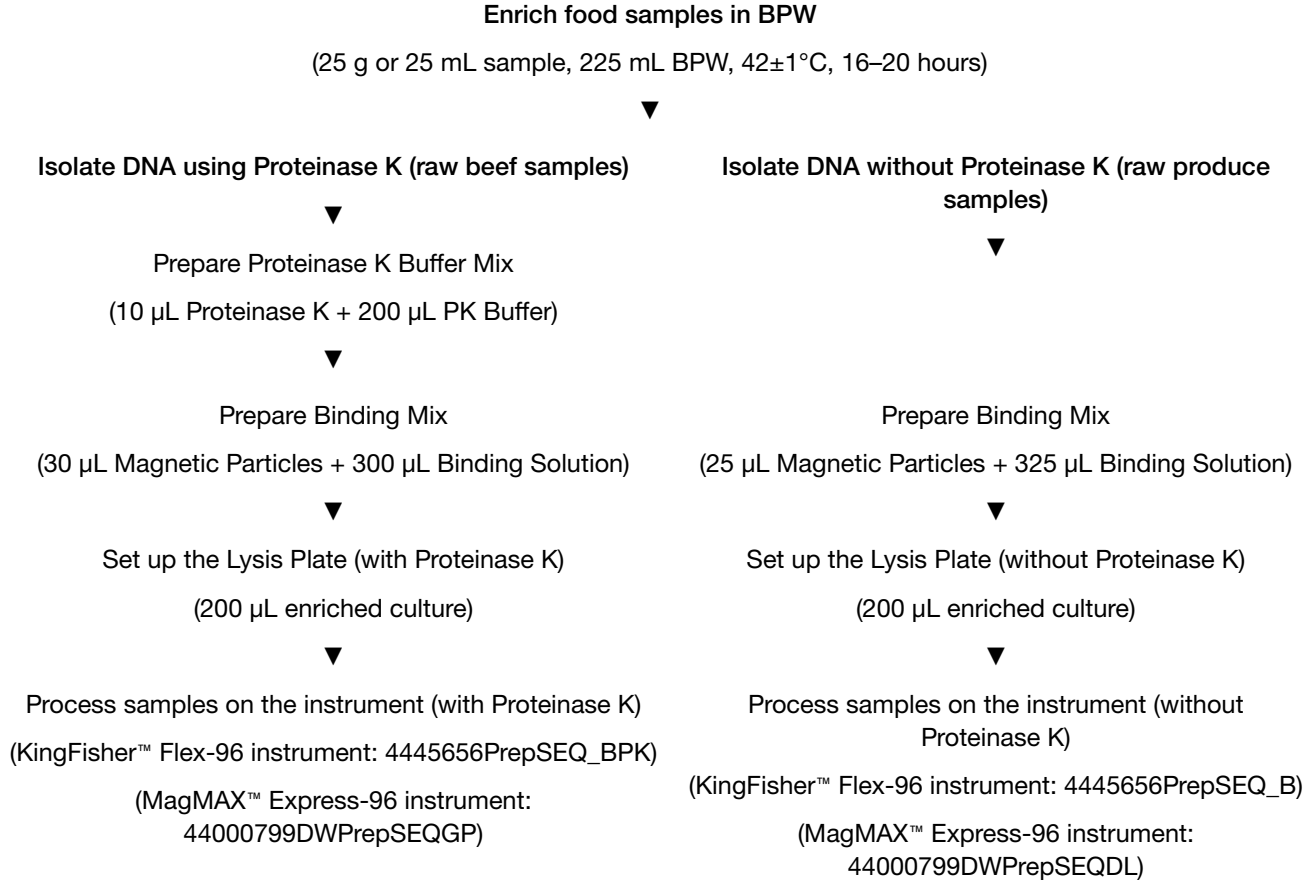
- At 5±3°C for up to 24 hours.
- Below –18°C for up to 1 year.

If required, validate storage of the DNA according to EN ISO 20837:2006.

# 3

## Enrich 25 g or 25 mL of food sample in BPW and isolate DNA (workflow B)

### Workflow



# Important procedural guidelines for enrichment and DNA isolation

## Guidelines for sample enrichment

- Use proper aseptic technique while handling samples to avoid cross-contamination.
- Use a forced air incubator and ensure sufficient space between enrichment bags to allow for air flow.

## For high-fat samples: remove fat layer before lysis

For samples that contain a distinct, top, fat layer following centrifugation, remove the fat layer and supernatant as follows:

Type of fat layer	Fat layer and supernatant removal
Liquid	<ol style="list-style-type: none"> <li>1. Use a P1000 pipettor to remove fat from the top surface by aspirating in a circular motion without disturbing the pellet.</li> <li>2. Continue to collect supernatant from the top surface until all the supernatant is removed.</li> <li>3. Discard the supernatant into a waste container.</li> </ol>
Solid	<ol style="list-style-type: none"> <li>1. Use a pipette tip to gently dislodge the fat layer without disturbing the pellet.</li> <li>2. Aspirate the supernatant from the top surface using a pipettor until all the supernatant is removed.</li> <li>3. Discard the supernatant into a waste container.</li> </ol>

## Enrich food samples in BPW

1. Prepare 225 mL of Buffered Peptone Water (BPW) for each 25 g or 25 mL of food sample, according to the manufacturer's instructions.
2. Combine the food sample with 225 mL of BPW in a homogenizer bag, and homogenize. A filtered bag may be used for enrichment of samples with particulates.

Sample type	Method
Coarse food types	Homogenize the sample thoroughly using a laboratory blender. Hand massage foods that cannot be processed in a homogenizer: squeeze the bag 5–10 times.
Liquids	Mix by hand.

3. Incubate the sample at 42±1°C under static conditions for 16–20 hours.

## Before first use of the kit

### Prepare Binding Solution and Wash Buffer

Before using a new PrepSEQ™ Nucleic Acid Extraction Kit, prepare the reagents:

- **Binding Solution**—Add approximately 35 mL of 100% isopropanol to an empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer**—Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, then mix well. Label the bottle to indicate that ethanol is added.

## Before each use of the kit

### Resuspend Magnetic Particles

---

**IMPORTANT!** Mix the particles vigorously before each use to ensure that all salts are dissolved.

White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is fully dissolved prior to use.

---

1. Incubate the tube of Magnetic Particles at  $37\pm 1^{\circ}\text{C}$  for approximately 10 minutes.
2. Vortex for approximately 10 seconds.

---

**Note:** If the white precipitate is not completely dissolved after 10 minutes at  $37^{\circ}\text{C}$ , apply longer incubation times and higher temperatures (up to  $50^{\circ}\text{C}$ ).

---

3. Keep at room temperature ( $23\pm 5^{\circ}\text{C}$ ) until ready for use.

## Set up the processing plates

Set up the processing plates as described in the following table.

Plate	Plate type	Action
Tip Comb	Standard	Place a 96-well deep-well tip comb in a standard plate.
Elution Plate	Standard	Add 140 $\mu\text{L}$ of Elution Buffer to each sample and control well.
Wash Plate 1	Deep-Well	Add 300 $\mu\text{L}$ of Wash Buffer to each sample and control well.
Wash Plate 2	Deep-Well	Add 300 $\mu\text{L}$ of Wash Buffer to each sample and control well.

## Isolate DNA using Proteinase K (raw beef samples)

### Prepare Proteinase K Buffer Mix

1. Combine the following components for the number of samples required.

Component	Volume per sample	Volume for $n$ samples <sup>[1]</sup>
Proteinase K, 20 mg/mL	10 $\mu$ L	11 $\mu$ L $\times n$
Proteinase K (PK) Buffer	200 $\mu$ L	220 $\mu$ L $\times n$

<sup>[1]</sup> Includes 10% overage.

2. Mix well, and use immediately or store on ice until ready to use.

### Prepare Binding Mix

Prepare Binding Mix just before use.

Combine the following components for the number of extractions required, and mix well by vortexing for approximately 10 seconds.

Component	Volume per extraction	Volume for $n$ samples <sup>[1]</sup>
Binding Solution (isopropanol)	300 $\mu$ L	330 $\mu$ L $\times n$
Magnetic Particles <sup>[2]</sup>	30 $\mu$ L	33 $\mu$ L $\times n$
<b>Total volume per extraction</b>	<b>330 <math>\mu</math>L</b>	<b>363 <math>\mu</math>L <math>\times n</math></b>

<sup>[1]</sup> Includes 10% overage.

<sup>[2]</sup> Resuspended and thoroughly mixed.

### Set up the Lysis Plate (with Proteinase K)

1. Transfer 200  $\mu$ L of enriched culture to a deep well Lysis Plate.
2. *(Optional but recommended)* Set up a negative extraction control (NEC) well containing 200  $\mu$ L of nuclease-free water.
3. Add 210  $\mu$ L of Proteinase K Buffer Mix to each sample and control well.

### Process samples on the instrument (with Proteinase K)

1. Select the program on the instrument, and press **Start**.

Instrument	Program
KingFisher™ Flex-96	4445656PrepSEQ_BPK
MagMAX™ Express-96	44000799DWPRepSEQGP

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the Tip Comb, then press <b>Start</b> .
Elution Plate	Load the Elution Plate, then press <b>Start</b> .
Wash Plate 2	Load Wash Plate 2, then press <b>Start</b> .
Wash Plate 1	Load Wash Plate 1, then press <b>Start</b> .
Lysis Plate	Load the Lysis Plate, then press <b>Start</b> .

3. Dispense 300  $\mu$ L of Lysis Buffer when prompted by the instrument (after 20 minutes).
- Remove the Lysis Plate and add 300  $\mu$ L of Lysis Buffer to each sample and control well.
  - Load the Lysis Plate into the instrument, and press **Start**.
4. Dispense 330  $\mu$ L of Binding Mix when prompted by the instrument (after 18 minutes).
- Vortex the Binding Mix for 5–10 seconds to ensure uniform distribution of the Magnetic Particles.
  - Remove the Lysis Plate and add 330  $\mu$ L of Binding Mix to each sample and control well.
  - Load the Lysis Plate into the instrument and press **Start**.

The remainder of the procedure (~30 minutes) is automated and does not require further user interaction.

5. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.

Proceed directly to real-time PCR. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film and store the DNA in one of the following ways:

- At  $5\pm 3^{\circ}\text{C}$  for up to 24 hours.
- Below  $-18^{\circ}\text{C}$  for up to 1 year.

If required, validate storage of the DNA according to EN ISO 20837:2006.



## Isolate DNA without Proteinase K (raw produce samples)

### Prepare Binding Mix

Prepare Binding Mix just before use.

Combine the following components for the number of extractions required, and mix well by vortexing for approximately 10 seconds.

Component	Volume per extraction	Volume for $n$ samples <sup>[1]</sup>
Binding Solution (isopropanol)	325 $\mu$ L	357.5 $\mu$ L $\times n$
Magnetic Particles <sup>[2]</sup>	25 $\mu$ L	27.5 $\mu$ L $\times n$
<b>Total volume per extraction</b>	<b>350 <math>\mu</math>L</b>	<b>385 <math>\mu</math>L <math>\times n</math></b>

<sup>[1]</sup> Includes 10% overage.

<sup>[2]</sup> Resuspended and thoroughly mixed.

### Set up the Lysis Plate (without Proteinase K)

1. Transfer 200  $\mu$ L of enriched culture to a deep well Lysis Plate.
2. *(Optional but recommended)* Set up a negative extraction control (NEC) well containing 200  $\mu$ L of nuclease-free water.
3. Add 300  $\mu$ L of Lysis Buffer to each sample and control well.

### Process samples on the instrument (without Proteinase K)

1. Select the program on the instrument, and press **Start**.

Instrument	Program
KingFisher™ Flex-96	4445656PrepSEQ_B
MagMAX™ Express-96	44000799DWPRepSEQDL

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the Tip Comb, then press <b>Start</b> .
Elution Plate	Load the Elution Plate, then press <b>Start</b> .
Wash Plate 2	Load Wash Plate 2, then press <b>Start</b> .
Wash Plate 1	Load Wash Plate 1, then press <b>Start</b> .
Lysis Plate	Load the Lysis Plate, then press <b>Start</b> .

3. Dispense 350  $\mu$ L of Binding Mix when prompted by the instrument (after 18 minutes).
  - a. Vortex the Binding Mix for 5–10 seconds to ensure uniform distribution of the Magnetic Particles.

- b. Remove the Lysis Plate from the instrument, and add 350  $\mu$ L of Binding Mix to each sample and control well.
- c. Load the Lysis Plate into the instrument and press **Start**.

The remainder of the procedure (~30 minutes) is automated and does not require further user interaction.

4. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.

Proceed directly to real-time PCR. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film and store the DNA in one of the following ways:

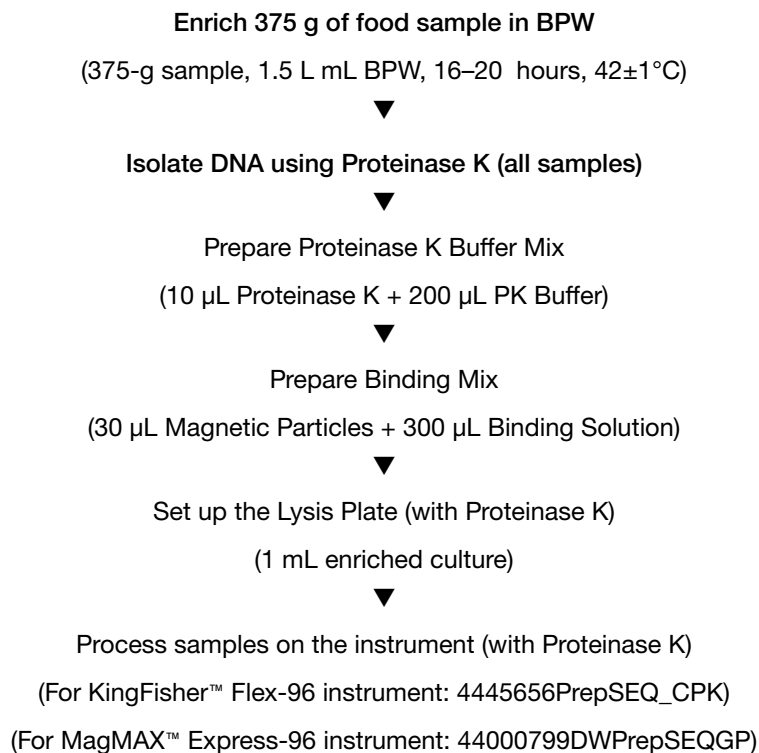
- At  $5\pm 3^{\circ}\text{C}$  for up to 24 hours.
- Below  $-18^{\circ}\text{C}$  for up to 1 year.

If required, validate storage of the DNA according to EN ISO 20837:2006.



# Enrich 375 g of food sample in BPW and isolate DNA (workflow C)

## Workflow



## Enrich 375 g of food sample in BPW

1. Prepare 1.5 L of Buffered Peptone Water (BPW) for each 375 g of food sample, according to the instructions of the manufacturer.
2. Combine the food sample with 1.5 L of BPW in a filtered homogenizer bag, and squeeze the bag 5–10 times to break up food chunks.
3. Incubate the sample at 42±1°C under static conditions for 16–20 hours.

## Before first use of the kit

### Prepare Binding Solution and Wash Buffer

Before using a new PrepSEQ™ Nucleic Acid Extraction Kit, prepare the reagents:

- **Binding Solution**—Add approximately 35 mL of 100% isopropanol to an empty Binding Solution bottle. Label the bottle to indicate that isopropanol is added.
- **Wash Buffer**—Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, then mix well. Label the bottle to indicate that ethanol is added.

## Before each use of the kit

### Resuspend Magnetic Particles

---

**IMPORTANT!** Mix the particles vigorously before each use to ensure that all salts are dissolved.

White precipitate occasionally forms in the Magnetic Particles tube. Extraction experiments show that formation of precipitate does not affect performance as long as the precipitate is fully dissolved prior to use.

---

1. Incubate the tube of Magnetic Particles at  $37\pm 1^{\circ}\text{C}$  for approximately 10 minutes.
2. Vortex for approximately 10 seconds.

---

**Note:** If the white precipitate is not completely dissolved after 10 minutes at  $37^{\circ}\text{C}$ , apply longer incubation times and higher temperatures (up to  $50^{\circ}\text{C}$ ).

---

3. Keep at room temperature ( $23\pm 5^{\circ}\text{C}$ ) until ready for use.

## Set up the processing plates

Set up the processing plates as described in the following table.

Plate	Plate type	Action
Tip Comb	Standard	Place a 96-well deep-well tip comb in a standard plate.
Elution Plate	Standard	Add 140 $\mu\text{L}$ of Elution Buffer to each sample and control well.
Wash Plate 1	Deep-Well	Add 300 $\mu\text{L}$ of Wash Buffer to each sample and control well.
Wash Plate 2	Deep-Well	Add 300 $\mu\text{L}$ of Wash Buffer to each sample and control well.

## Isolate DNA using Proteinase K (all samples)

### Prepare Proteinase K Buffer Mix

1. Combine the following components for the number of samples required.

Component	Volume per sample	Volume for $n$ samples <sup>[1]</sup>
Proteinase K, 20 mg/mL	10 $\mu$ L	11 $\mu$ L $\times n$
Proteinase K (PK) Buffer	200 $\mu$ L	220 $\mu$ L $\times n$

<sup>[1]</sup> Includes 10% overage.

2. Mix well, and use immediately or store on ice until ready to use.

### Prepare Binding Mix

Prepare Binding Mix just before use.

Combine the following components for the number of extractions required, and mix well by vortexing for approximately 10 seconds.

Component	Volume per extraction	Volume for $n$ samples <sup>[1]</sup>
Binding Solution (isopropanol)	300 $\mu$ L	330 $\mu$ L $\times n$
Magnetic Particles <sup>[2]</sup>	30 $\mu$ L	33 $\mu$ L $\times n$
<b>Total volume per extraction</b>	<b>330 <math>\mu</math>L</b>	<b>363 <math>\mu</math>L <math>\times n</math></b>

<sup>[1]</sup> Includes 10% overage.

<sup>[2]</sup> Resuspended and thoroughly mixed.

### Set up the Lysis Plate (with Proteinase K)

1. Transfer 1 mL of enriched culture to a 1.5-mL microcentrifuge tube, then centrifuge the tube at 12,000–16,000  $\times g$  for about 3 minutes.
2. Remove and discard the supernatant as quickly as possible to prevent dissipation of the pellet.

---

**Note:** If no pellet is visible after centrifugation (for example, as found in filtered juices), leave ~50  $\mu$ L of supernatant in the tube to avoid aspiration the bacterial pellet.

---

3. (Optional) If necessary, follow “For high-fat samples: remove fat layer before lysis” on page 14.
4. Add 210  $\mu$ L of Proteinase K Buffer Mix to the pellet, and mix well to resuspend the pellet.
5. Transfer the sample to a deep well Lysis Plate.
6. (Optional but recommended) Set up a negative extraction control (NEC) well containing 210  $\mu$ L of nuclease-free water.

## Process samples on the instrument (with Proteinase K)

1. Select the program on the instrument, and press **Start**.

Instrument	Program
KingFisher™ Flex-96	4445656PrepSEQ_CPK
MagMAX™ Express-96	44000799DWPRepSEQGP

2. Load the prepared plates according to the readout on the instrument, verifying that their orientation is {A1 to A1}.

Plate	Action
Tip Comb	Load the Tip Comb, then press <b>Start</b> .
Elution Plate	Load the Elution Plate, then press <b>Start</b> .
Wash Plate 2	Load Wash Plate 2, then press <b>Start</b> .
Wash Plate 1	Load Wash Plate 1, then press <b>Start</b> .
Lysis Plate	Load the Lysis Plate, then press <b>Start</b> .

3. Dispense 300 µL of Lysis Buffer when prompted by the instrument (after 20 minutes).
  - a. Remove the Lysis Plate and add 300 µL of Lysis Buffer to each sample and control well.
  - b. Load the Lysis Plate into the instrument, and press **Start**.
4. Dispense 330 µL of Binding Mix when prompted by the instrument (after 18 minutes).
  - a. Vortex the Binding Mix for 5–10 seconds to ensure uniform distribution of the Magnetic Particles.
  - b. Remove the Lysis Plate and add 330 µL of Binding Mix to each sample and control well.
  - c. Load the Lysis Plate into the instrument and press **Start**.

The remainder of the procedure (~30 minutes) is automated and does not require further user interaction.

5. When DNA sample preparation is complete (“Enjoy your DNA” is displayed on the screen), remove the Elution Plate from the instrument.

Proceed directly to real-time PCR. Alternatively, seal the plate with MicroAmp™ Clear Adhesive Film and store the DNA in one of the following ways:

- At 5±3°C for up to 24 hours.
- Below –18°C for up to 1 year.

If required, validate storage of the DNA according to EN ISO 20837:2006.



# Perform PCR with the MicroSEQ™ E. coli O157:H7 Detection Kit and RapidFinder™ Express Software

## Important procedural guidelines for PCR

### Software

RapidFinder™ Express Software determines the Run Layout (plate layout) during creation of the run file, therefore it must be set up before distributing DNA samples to the assay beads.

For additional information, refer to the *Applied Biosystems™ RapidFinder™ Express Software Quick Reference* (Pub. No. 4480999) or the online help within the software.

### Sample handling

- If DNA samples were stored before PCR, thaw (if necessary), vortex, then centrifuge at 1,000–2,000 × *g* for approximately 1 minute to remove any condensation from the adhesive film before opening the plate (to avoid cross contamination).
- Use a new pipette tip for each sample.
- If you mix the assay beads with the DNA samples by pipetting up and down, keep the pipette tip at the bottom of the tube to minimize aerosol formation and cross-contamination.
- Follow the recommendations in “Good laboratory practices for PCR” on page 41.

### Avoid fat layer and particulates after sample lysis (collection of DNA sample for PCR)

If you see this in the Elution Plate...	Do this...
Oil droplets as a top layer	After lysis, food samples with high fat or oil content can form a top layer containing fat and debris over the aqueous phase containing the DNA. Collect the DNA sample for PCR from the clear middle phase, avoiding the top layer and bottom pellet (See Figure 1).
Magnetic Particles	<ol style="list-style-type: none"><li>1. Place the Elution Plate on a 96-well magnetic ring stand for at least 1 minute.</li><li>2. Collect the eluate for PCR while the Elution Plate remains on the magnetic stand. Avoid touching the Magnetic Particles.</li></ol>

(continued)

If you see this in the Elution Plate...	Do this...
Particulate residue from food sample	If the particulate residue is not removed using a 96-well magnetic ring stand: <ol style="list-style-type: none"> <li>1. Centrifuge the Elution Plates at about 4000 × g for about 30 seconds in a plate centrifuge.</li> <li>2. Avoid the particulate residue, and collect eluate for PCR.</li> </ol>

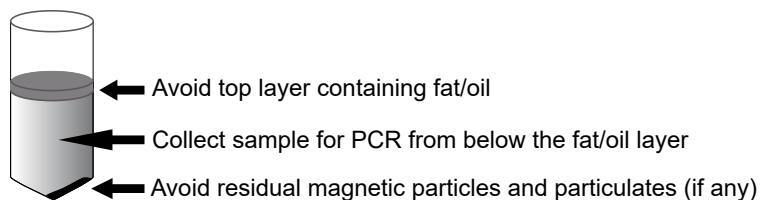


Figure 1 High-fat samples: Collect sample from middle phase after lysis.

## MicroAmp™ tube strips

- Follow these instructions to ensure proper storage of the tube strips:
  - Cut the storage pouch at the notch above the resealable strip.
  - Always reseal the storage pouch with desiccant, and replace at 5±3°C.
- 8-tube strips can be cut apart with scissors.  
If necessary, trim any remaining connector material from the cut to allow a better fit against adjacent tubes in the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips.
- MicroAmp™ Tube Strips are labeled 1–8 on the side of the tubes to orient tube strips during handling.

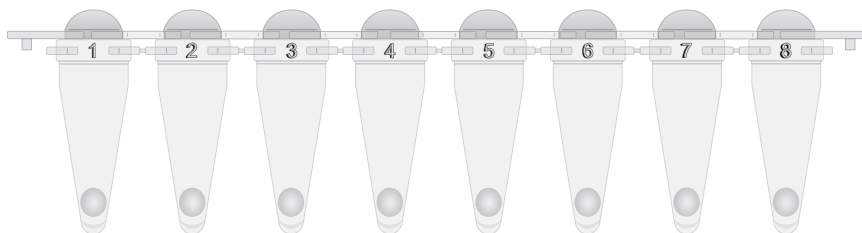


Figure 2 MicroAmp™ Tube Strip labeling The tube strip is shown with tinted dome caps, as shipped. For PCR, replace the dome caps with the optical cap strips provided in the kit.


If necessary for visual reference from above, mark the tab at one end of the cap strip. Do not mark any of the caps (this could interfere with real-time PCR detection).

- Seal the tubes with the transparent, optical cap strips provided in the kit. Do not use colored caps or tubes for real-time PCR reactions, because they may affect dye-signal readings during real-time PCR.
- Always use intact 8-cap strips, even if empty tubes have been added next to reaction tubes.



- Use the MicroAmp™ 96-Well Base and the MicroAmp™ Cap Installing Tool to seal the assay tubes with the optical cap strips. This avoids collapsing, bending, or misaligning the tubes. Confirm that the strips are straight and that each tube is in line with the adjacent tube.
- Use a plate adapter for vortexing the tube strips, or hold the strips in the MicroAmp™ 96-Well Base while vortexing.

## Create or edit a run file in RapidFinder™ Express Software

On the main page of the RapidFinder™ Express Software, select **Create/Edit a Run File** , then select the target pathogen, number of samples, replicates, and positive and negative controls for each target at the prompts.

The software determines the sample layout based on the information entered and creates a run file.

## Prepare the assay beads

Follow the plate layout determined by the RapidFinder™ Express Software.

1. Transfer the appropriate number of individual tubes or 8-tube strips from the storage pouch to a 96-well base at room temperature ( $23\pm 5^{\circ}\text{C}$ ).
2. If required by the plate layout, place empty MicroAmp™ Fast 8-Tube Strips (or partial strips) to balance the tray when the assay tubes are placed in the instrument later.

## Set up the PCR reactions

For step-by-step instructions, select **Pipette Samples**  on the main page in RapidFinder™ Express Software.

1. If necessary, thaw samples and controls completely, and mix each sample or control thoroughly. If the Elution Plate contains oil droplets, magnetic particles, or food particulate residue, see “Avoid fat layer and particulates after sample lysis (collection of DNA sample for PCR)” on page 31. If the DNA samples have been stored, see “Sample handling” on page 31.
2. Following the layout determined by RapidFinder™ Express Software, add 30  $\mu\text{L}$  of sample or control to each assay bead at room temperature ( $23\pm 5^{\circ}\text{C}$ ), and mix by gently pipetting up and down a few times.  
Beads dissolve in 1–5 seconds.  
Alternatively, vortex the assay tubes after they are capped in the final step.
3. Seal the tubes with the transparent, optical cap strips provided in the kit.
4. Ensure that the reactions are thoroughly mixed: if reactions were not previously mixed during the pipetting step, vortex at high speed for 5–10 seconds.

5. Ensure that the reagents are at the bottom of tubes: briefly centrifuge the tube strips at  $200\text{--}600 \times g$  for about 20 seconds.

---

**IMPORTANT!** If needed, repeat the vortex/centrifugation steps to ensure complete mixing of the samples with the assay beads.

---

## Load and run the reactions

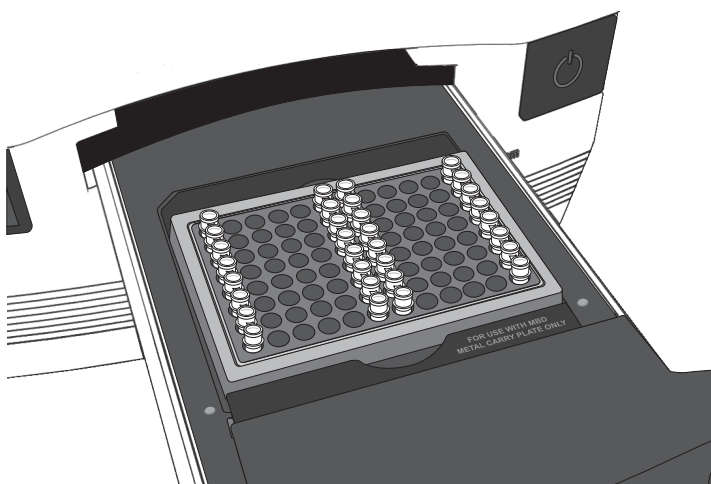
In the RapidFinder™ Express Software, select **Start Instrument Run**  on the main page, select the appropriate run file, and follow the software prompts.

1. Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout.

Use the 7500 Fast Precision Plate Holder for MicroAmp™ Tube Strips in the instrument.

Be sure to load empty low profile PCR tubes as directed by the software (Figure 3).


2. Close the tray to the instrument, and follow the RapidFinder™ Express Software prompts to start the run.



**Figure 3 7500 Fast instrument tube layout** RapidFinder™ Express Software directs the user to load empty strip tubes in column 1 (far left) and column 12 (far right), if needed. The empty capped 8-tube strips evenly distribute the clamping load applied to the sample tube strips during processing, thereby minimizing the risk of collapsing any tubes.

## View results and data analysis

Data analysis is automated by the software.

In the RapidFinder™ Express Software, select **View Results**  on the main page, select the appropriate run file, and follow the prompts to view results.

To display a list of results in table format, click **Table View**. Select a sample, then click **View Details** to see replicate information about samples.



## Recommended confirmation methods

In the context of AOAC Research Institute *Performance Tested Methods*<sup>SM</sup> certification, enriched cultures with positive PCR results were tested further by cultural confirmation using the appropriate reference method for the sample matrix (see “*AOAC Performance Tested Methods*<sup>SM</sup> Certification” on page 40).



# Troubleshooting

Observation	Possible cause	Recommended action
A visual difference in PCR beads is observed.	PCR pellets can exhibit differences in morphology.	Ensure thorough pipette mixing followed by vortexing on high speed to confirm pellet is in solution. After PCR, if IPC failure is observed, repeat the reaction.
Bacterial pellet is difficult to avoid during removal of supernatant	The sample was left unattended before removal of the supernatant, causing dissipation of the bacterial pellet.	Remove the supernatant immediately following centrifugation.
	The size of the bacterial pellet is very small and difficult to see.	Remove the supernatant carefully, leaving behind up to 50 $\mu$ L of supernatant to avoid aspiration of the pellet.
Bacterial pellet is difficult to resuspend during lysis	Pellet is too hard.	Ensure maximum resuspension of the pellet in the Lysis Buffer or Proteinase K Buffer Mix before proceeding.
		Transfer the entire contents, including the incompletely resuspended pellet (if any), to the Lysis Plate.
Inhibition of downstream PCR, indicated by nondetection of IPC reaction	Magnetic Particles were in the Elution Plate.	<p>Avoid disturbing the Magnetic Particles during transfer of eluted DNA to the lyophilized assay.</p> <p>Avoid transfer of Magnetic Particles using one of the following methods (<i>optional</i>):</p> <ul style="list-style-type: none"> <li>Place the Elution Plate on the 96-Well Magnetic Ring Stand during transfer of eluted DNA sample to the lyophilized assay.</li> <li>Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 <math>\times</math> <i>g</i> for approximately 30 seconds to pellet the Magnetic Particles to the bottom of the plate.</li> </ul>
	Elution Plate contained incompletely removed particulate residue from the food sample.	<p>Avoid residue during transfer of eluted DNA to the lyophilized assay.</p> <p>(<i>Optional</i>) Spin the plate at maximum speed in a plate centrifuge for the equivalent of approximately 4,000 <math>\times</math> <i>g</i> for approximately 30 seconds to pellet the food residue to the bottom of the plate.</p>

Observation	Possible cause	Recommended action
Inhibition of downstream PCR, indicated by nondetection of IPC reaction (continued)	For samples of enriched culture that were centrifuged before lysis, the removal of sample supernatant before addition of lysis buffer was incomplete.	Ensure maximal removal of the supernatant without disturbing the bacterial pellet.
In positive control wells, no target-specific signal is detected.	Positive control was omitted (pipetting error).	Repeat the assay. Make sure to pipette the positive control into all positive control wells.
In positive control wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In negative extraction control wells, target-specific signal is detected. The result is considered invalid by the software.	Carryover contamination occurred.	<ol style="list-style-type: none"> <li>1. Repeat the assay using fresh aliquots of all reagents, fresh enrichment, and clean pipetting equipment.</li> <li>2. If the negative extraction control continues to show contamination, repeat the assay using a new kit.</li> <li>3. If the negative extraction control continues to show contamination, contact Technical Support.</li> </ol>
In negative control wells, no IPC signal is detected, but a target-specific signal is detected	Carryover contamination caused target signal in negative control wells.  Additionally, no IPC signal in negative control wells could be caused by: <ul style="list-style-type: none"> <li>• A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA.</li> <li>• A problem occurred with IPC amplification.</li> </ul>	To correct carryover contamination, repeat the assay using fresh aliquots of all reagents and clean pipetting equipment.
		To determine whether IPC amplification is a problem, examine unknown wells for an IPC signal. If an IPC signal is present, IPC amplification is not a problem.
In unknown wells, no IPC or target-specific signal is detected.	Inhibition of PCR occurred.	Dilute the sample 1:5 with nuclease-free water to dilute PCR inhibitors, then repeat the assay. If PCR remains inhibited, repeat the sample preparation.  Refer to other troubleshooting suggestions for removal of Magnetic Particles or particulate residue from the DNA sample.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.

Observation	Possible cause	Recommended action
Multicomponent plot signals for FAM™, VIC™, and ROX™ detectors increase/decrease during cycles 1–15, but the amplification curve and result are not affected (this observation applies to <b>View in SDS mode</b> ).	Incomplete mixing and dissolution of the lyophilized bead with sample or control occurred.	<p>After adding 30 µL of sample or Pathogen Negative Control to the bead and capping the tubes:</p> <ol style="list-style-type: none"> <li>Vortex strips at high speed for about 10 seconds, then centrifuge the strips at 200–600 × <i>g</i> for about 10 seconds.</li> <li>Vortex the strips again on high speed for about 10 seconds, then centrifuge the strips at 200–600 × <i>g</i> for about 1 minute.</li> </ol> <p>Ensure that all liquid is at the bottom of the tubes and the beads are fully dissolved before proceeding.</p>
Replicate results for a sample are inconsistent.	All replicate wells for a sample did not have the same result.	<p>If more than two replicates yield the same result (for example, 2 of 3 replicates are negative, but 1 replicate is positive), refer to your laboratory protocol to determine whether to repeat the assay using fresh samples and reagents.</p> <p>If only 2 replicates were run and the results are not consistent, repeat the assay using fresh samples and reagents.</p>

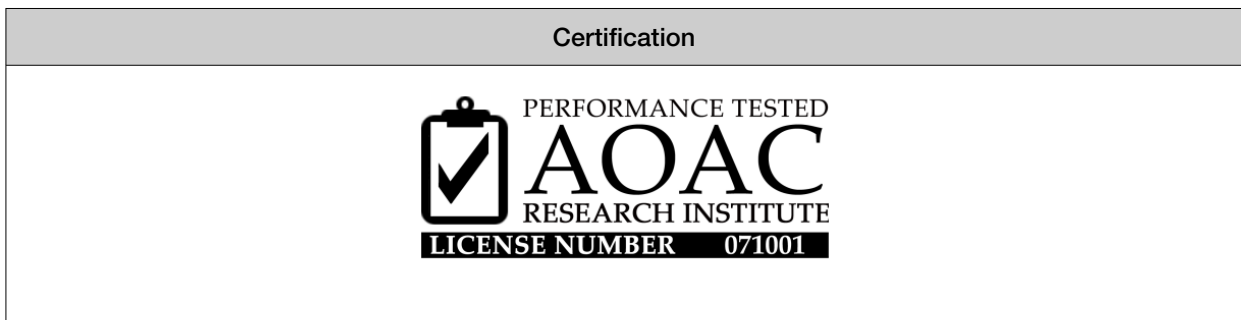
Observation	Possible cause	Recommended action
<p>Amplicon contamination.</p>	<ul style="list-style-type: none"> <li>• Contamination was introduced into the PCR clean area from post-amplification reaction tubes that were either opened in the clean area or brought into the PCR clean area from contaminated gloves or solutions.</li> <li>• Contamination was introduced into the real-time PCR instrument from crushed and broken PCR reaction tubes.</li> </ul>	<p>To confirm amplicon contamination, perform the following experiment:</p> <p>Prepare negative control samples using at least one 8-tube strip of MicroSEQ™ Assay Beads.</p> <ol style="list-style-type: none"> <li>1. Divide the assay beads into two sets.               <ol style="list-style-type: none"> <li>a. To the first set of assay beads, add 30 µL of nuclease-free water.</li> <li>b. To the second set of assay beads, add 29 µL of nuclease-free water plus 1 µL of 1 U/µL Uracil DNA Glycosylase (Cat. No. 18054-015).</li> </ol> </li> <li>2. Run samples on the 7500 Fast Real-Time PCR Instrument using SDS software, then select <b>Fast 7500</b> run mode.</li> <li>3. Under the instrument tab:               <ul style="list-style-type: none"> <li>• Select <b>Add Step</b> to stage 1 of the PCR cycle that consists of 10 minutes at 50°C.</li> <li>• Extend the 95°C step from 20 seconds to 10 minutes.</li> </ul> </li> </ol> <p>Amplicon contamination is indicated by target-specific signal in the –UNG samples and no target-specific signal in +UNG samples.</p> <p>If the instrument block was contaminated, consult the <i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve</i> (Pub. No. 4347825) and/or contact a service representative to clean the instrument.</p>



# Supplemental information

## AOAC Performance Tested Methods<sup>SM</sup> Certification

Table 8 Performance Tested Methods<sup>SM</sup> Certification of the workflow



The detection of *E. coli* O157:H7 using PrepSEQ<sup>TM</sup> Nucleic Acid Extraction Kit and the MicroSEQ<sup>TM</sup> E. coli O157:H7 Detection Kit has earned the AOAC Performance Tested Methods<sup>SM</sup> Certification from the AOAC Research Institute. The certified workflow described in this user guide includes:

- Enrichment in BHI or BPW
- PrepSEQ<sup>TM</sup> Nucleic Acid Extraction Kit
- MicroSEQ<sup>TM</sup> E. coli O157:H7 Detection Kit
- Applied Biosystems<sup>TM</sup> 7500 Fast Real-Time PCR Instrument
- RapidFinder<sup>TM</sup> Express Software v2.0 or later
- Applied Biosystems<sup>TM</sup> 7500 Fast Real-Time PCR Instrument and equivalents manufactured by Thermo Fisher Scientific and/or subsidiaries (see Table 9 for characteristics) with RapidFinder<sup>TM</sup> Express Software v2.0 or later.

Table 9 7500 Fast Real-Time PCR Instrument characteristics

Characteristics	7500 Fast Real-Time PCR Instrument
Optics	12v 75w halogen bulb
Filters	5 excitation and 5 emission filters
Sample ramp rate	Standard mode: $\pm 1.6^{\circ}\text{C}/\text{sec}$ Fast mode: $\pm 3.5^{\circ}\text{C}/\text{sec}$
Thermal range	4-100 $^{\circ}\text{C}$
Thermal accuracy	$\pm 0.5^{\circ}\text{C}$



Table 9 7500 Fast Real-Time PCR Instrument characteristics (continued)

Characteristics	7500 Fast Real-Time PCR Instrument
Thermal uniformity	±1°C
Format	96-well, 0.1-mL block

- Confirmation testing of positive samples as described in Chapter 6, “Recommended confirmation methods”.

Table 10 Validated matrices

Reference method	Matrix
USDA MLG 5.04	<ul style="list-style-type: none"> <li>• 25 g of ground beef and beef trim</li> <li>• 375 g of ground beef and beef trim</li> </ul>
ISO 16654 (2001)	<ul style="list-style-type: none"> <li>• 25 g of spinach</li> <li>• 25 g of apple juice</li> <li>• 25 g of orange juice</li> </ul>

Go to [thermofisher.com/foodsafety](http://thermofisher.com/foodsafety) for a list of workflows for detection of *E. coli* (Pub. No. MAN0009419).

## Good laboratory practices for PCR

**Note:** Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.

- Clean lab benches and equipment periodically with 10% bleach solution or DNAZap™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2005 or [www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html](http://www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html).



**WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).

## Chemical safety



**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



## Biological hazard safety



**WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at:  
[www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf](http://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf)
- World Health Organization, *Laboratory Biosafety Manual*, 4th Edition, WHO/CDS/CSR/LYO/2020.12; found at:  
[www.who.int/publications/i/item/9789240011311](http://www.who.int/publications/i/item/9789240011311)



# Documentation and support

## Food safety support

Website: <https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html> or [thermofisher.com/foodsafety](https://www.thermofisher.com/foodsafety)

Support email:

- Europe, Middle East, Africa: [microbiology.techsupport.uk@thermofisher.com](mailto:microbiology.techsupport.uk@thermofisher.com)
- North America: [microbiology@thermofisher.com](mailto:microbiology@thermofisher.com)

Phone: Visit [thermofisher.com/support](https://www.thermofisher.com/support), select the link for phone support, then select the appropriate country from the dropdown list.

## Customer and technical support

Visit [thermofisher.com/support](https://www.thermofisher.com/support) for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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## Related documentation

Document	Publication number
<i>RapidFinder™ Express Software Quick Reference</i>	4480999
<i>Thermo Scientific™ KingFisher™ Flex User Manual</i>	N07669

*(continued)*

Document	Publication number
<i>Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide</i>	4378657
<i>Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide</i>	4387777
<i>PCR Starter Kit for 96-well blocks, 0.2 mL, User Guide</i>	A24829

# References

EN ISO 16654:2001. Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *E. coli* O157.

EN ISO 22174:2005. Microbiology of food and animal feeding stuffs—Polymerase chain reaction (PCR) for the detection of food-borne pathogens—General requirements and definitions.

Food Safety and Inspection Service (USDA). 2008. Detection, isolation and identification of *Escherichia coli* O157:H7 from meat products. MLG 5.04. Microbiology Laboratory Guidebook.

